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A Review on application of Nanoscience for Biosensing

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Abstract : ***Nanotechnology is playing an increasingly important role in the development of biosensors. The sensitivity and performance of biosensors is being improved by using nanomaterials for their construction. The use of these nanomaterials has allowed the introduction of many new signal transduction technologies in biosensors. Because of their submicron dimensions, nanosensors,nanoprobes and other nano systems have allowed simple and rapid analyses in vivo. Portable instruments capable of analyzing multiple components are becoming available. This work reviews the status of the various nanostructure-based biosensors. Use of the self-assembly techniques and nano-electromechanical systems (NEMS) in biosensors is discussed.***

Keywords: Biosensors; Nanotechnology; Nanomaterials; Nanostructure; Nano-electromechanical systems (NEMS); Self-assembly.

I. Introduction

1.Nanotechnology involves the study, manipulation, creation and use of materials,devices and systems typically with dimensions smaller than 100 nm. Nanotechnology is playing an increasingly important role in the development of biosensors **(Vo-Dinh et al.,**
2001; Haruyama, 2003; Jain, 2003). Sensitivity and other attributes of biosensors can be improved by using nanomaterials in their construction. Nanomaterials, or matrices with at least one of their dimensions ranging in scale from 1 to 100 nm, display unique physical and chemical features because of effects such as the quantum size effect, mini size effect, surface effect and macro-quantum tunnel effect. Use of nanomaterials in biosensors allows the use of many new signal transduction technologies in their manufacture. Because of their submicron size, nanosensors, nanoprobes and other nanosystems are revolutionizing the fields of chemical and biological analysis, to enable rapid analysis of multiple substances in vivo. Here we review the major aspects of the nanotechnology-based biosensors.

II.Application of Nano Material in Biosensing

Nanostructures

Novel nanomaterials for use in bioassay applications represent a rapidly advancing field. Various nanostructures have been investigated to determine their properties and possible applications in biosensors. These structures include nanotubes, nanofibers, nanorods, nanoparticles and thin films. Of these, nanoparticles are the best studied. The biosensors based on different kinds of nanostructures are discussed in this review.

2.1. Nanoparticles in biosensing

Nanoparticles have numerous possible application in biosensors. For example, functional nanoparticles (electronic, optical and magnetic) bound to biological molecules (e.g. peptides, proteins, nucleic acids) have been developed for use in biosensors to detect and amplify various signals. Some of the nanoparticle-based sensors include the acoustic wave biosensors, optical biosensors, magnetic and electrochemical biosensors, as discussed next.

2.1.1. Acoustic wave biosensors

Acoustic wave biosensors were developed to greatly improve the sensitivity and limits of detection **(Ward and Ebersole, 1996**). In the mass-amplified quartz crystal microbalance assay variant of this technology, antibody modified sol particles indirectly bind to an electrode surface by complexing to an analyte that has been itself captured by an antibody immobilized on the electrode surface. The large mass of the bound sol particles greatly affects the vibrational frequency of the quartz crystal and this is used as the basis for detection. The assay can be carried out in the competitive mode. The preferred diameter of sol particles is in the range of 5–100 nm. Other high-density particles (e.g. Au, Pt, CdS, TiO_2, polymers) may be also suitable **(Su et al., 2000; Liu et al., 2004).**

2.1.2. Optical biosensors

Resonance enhancement of metal nanoclusters bound to a surface by biorecognitive interactions has been reported as effective for use in bio-optical sensory devices **(Bauer etal., 1999).** Lectin–sugar, antigen–antibody and protein–receptor interactions have been employed in these assays. The analytes induced binding or dissociation of metal nanoclusters, located a defined distance from a reflecting and preferably electron-conducting substrate surface. The binding or dissociation could be transduced into a clearly detectable optical signal through resonant enhancement of clusters interacting with their mirror dipoles. Gold nanoparticles have been used as a new class of universal fluorescence quenchers to develop an optical biosensor for recognizing and detecting specific DNA sequences **(Maxwell et al., 2002**). Attached to gold nanoparticles were oligonucleotide molecules labeled with a thiol group at one end and a fluorophore at the other end. This hybrid bio/ inorganic construct was found to spontaneously assemble into a constrained arch-like conformation on the nanoparticle surface. Binding of target molecules resulted in a conformation change and this restored the fluorescence of the quenched fluorophore. The biosensor developed on this basis was able to detect single-base mutations in a homogeneous format.

2.1.3. Magnetic biosensors

Magnetic nanoparticles are a powerful and versatile diagnostic tool in biology and medicine. They usually can be prepared in the form of either single domain or superparamagnetic (Fe_3O_4), greigite (Fe_3S_4), maghemite (g-Fe_2O_3), and various types of ferrites (MeO_Fe_2O_3, where Me = Ni, Co, Mg, Zn, Mn, etc.). Bound to biorecognitive molecules, magnetic nanoparticles can be used to separate or enrich the analyte to be detected. Established techniques such as magnetic cell separation use magnetic field gradients to manipulate and isolate magnetically labeled cells **(Sˇafarˇık and Sˇafarˇıkova´, 1999**). Magnetic

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immunoassay techniques also have been developed in which a magnetic field generated by the magnetically labeled targets is detected directly with a magnetometer **(Richardson et al., 2001)**.

A new technique has been introduced for rapid detection of biological targets by using superparamagnetic nanoparticles and a ''microscope'' based on a high-transition temperature dc superconducting quantum interference device (SQUID) **(Chemla et al., 2000)**. In this technique, a mylar film with bound targets is placed on the microscope. A suspension of magnetic nanoparticles carrying antibodies is added to the mixture in a well, and 1-s pulses of magnetic field are applied parallel to the SQUID. In the presence of this aligning field, the nanoparticles develop a net magnetization, which relaxes when the field is turned off. Unbound nanoparticles relax rapidly by Brownian rotation and contribute no measurable signal. Nanoparticles bound to the target are captured and undergo Ne¨el
relaxation, producing a slowly decaying magnetic flux, which is detected by the SQUID. The ability to distinguish between bound and unbound labels allows anyone to run homogeneous assays, which do not require separation and removal of unbound magnetic particles. Magnetic nanoparticles or microspheres have been reviewed in detail **by Ha¨feli et al. (1997)** and **S ˇ afarˇık and S ˇ afarˇıkova´ (2002).**

2.1.4. Electrochemical biosensors

Electrochemical biosensors have been fabricated from mostly metallic nanoparticles. Metal nanoparticles based electroanalysis has been reviewed by **Herna´ndez-Santos et al. (2002**). Metal nanoparticles can be used to enhance the amount of immobilized biomolecules in construction of a sensor. Because of its ultrahigh surface area, colloidal Au has been used to enhance the DNA immobilization on a gold electrode, to ultimately lower the detection limit of the fabricated electrochemical DNA biosensor **(Cai et al., 2001**). Self-assembly of approximately 16-nm diameter colloidal Au onto a cysteamine modified gold electrode
resulted in an easier attachment of an oligonucleotide with a mercaptohexyl group at the 5V-phosphate end and increased the capacity for nucleic acid detection. Quantitative results showed that the surface densities of oligonucleotides on the Au colloid modified gold electrode were approximately $1\_4\_10^{14}$ molecules cm^{-2}. The detection limit was 5_10^{-10} mol_l^{-1} of complementary ssDNA. Metal nanoparticles have been used to catalyze biochemical reactions and this capability can be usefully employed in biosensor design. Catalysis is the most important
and widely used chemical application of metal nanoparticles and has been studied extensively. Transition metals, specially precious metals, show very high catalytic abilities for many organic reactions. Nanoparticles behave in the reaction medium as do conventional homogeneous catalysts, but can be easily recovered after the reaction. Enzyme–gold colloids have been used on the surface of electrodes to fabricate biosensors for H_2O_2, glucose, xanthine and hypoxanthine **(Crumbliss et al., 1992; Zhaoet al., 1996; Xu et al., 2003). Xu et al. (2003)** studied the electrochemistry of horseradish peroxidase (HRP) immobilized on a colloidal gold modified screen-printed carbon electrode. The immobilized HRP displayed fast amperometric response and an electrocatalytic activity to the reduction of hydrogen peroxide (H_2O_2) without the aid of an electron mediator. The biosensor exhibited high sensitivity, good reproducibility and longterm stability for the determination of H_2O_2 with a linear range from 0.8 AM to 1.0 mM and a detection limit of 0.4 AM. Nanosized semiconductor crystals can also increase efficiency of photochemical reactions and can be effectively coupled to biomolecular units such as enzyme, to generate novel photoelectrochemical systems **(Curri et al., 2002). Curri et al. (2002)** immobilized nanocrystalline CdS by self-assembly onto a gold electrode in order to prepare, in combination with formaldehyde dehydrogenase (FDH) enzyme, a biological-inorganic hybrid that was able to carry out the catalytic oxidation of formaldehyde. The preliminary results indicated that quantum-sized CdS layer on gold, in close contact with the enzyme, was an effective photoactive material for replacing the NAD^+/NADH needed as charge transfer molecule in the enzymatic reaction. Metal nanoparticles have been used to facilitate the electron transfer in nanoelectronic devices. Gold nanoparticles can greatly improve electron transfer across the monolayer molecules self-assembled on the surfaces of electrodes **(Zhang et al., 2001).** This observation may be specially useful in the development of electroluminescene-based biosensors. Metal nanoparticles can be used as an electrochemical label. Most biological molecules can be labeled with metal nanoparticles without compromising their biological activities. Affinity assays can then be performed by monitoring the electrochemical signal of these metal nanoparticles. A new electrochemical method for monitoring biotin–streptavidin interaction has been developed. This is based on the use of colloidal gold as an electrochemical label **(Gonzalez-Garcia et al., 2000**). Biotinylated albumin is adsorbed on the pretreated surface of a carbon paste electrode. This modified electrode is immersed in the colloidal gold– streptavidin labeled solution. Adsorptive voltammetry is used to monitor colloidal gold bound to streptavidin. The analytical signal is highly reproducible. A linear relationship between the peak current and streptavidin concentration from 2.5 nM to 25 AM was obtained for a sequential competitive assay. **Kim et al. (2000)** developed a disposable immunochromatographic sensor for on-line quantitative determination of human serum albumin (HSA). The sensor used conductimetric detection and 20 nm gold colloid particles modified with polyaniline (a conducting polymer) for signal generation **(Kim et al., 2000).** The immunoassay was carried out in a membrane strip sensor with two interdigitated silver electrodes that were screen-printed on a nitrocellulose membrane. The immuno-strips were placed in the analyte solution in an erect position and the solution was absorbed from the bottom of the strips. The reaction between the conjugate and analyte took place immediately and this complex was carried up into the next membrane that had the immobilized antibody. The second antigen_antibody reaction formed a sandwich-type immune complex at the electrode and polyaniline-bound colloidal gold generated a conductimetric signal. A novel array-based electrical detection of DNA with nanoparticle probes was reported by **Park et al. (2002)**. Capture strands of alkylthiol-modified oligonucleotides were immobilized onto the activated surface of SiO2 substrate between two ends of Au microelectrodes with 20 Am gaps. The binding events localized gold nanoparticles in the electrode gap. Silver deposition facilitated by the gold nanoparticles bridged the gap and led to readily measurable conductivity changes. The method could be used to detect target DNA at concentrations as low as 500 fM with a point mutation selectivity factor of f100,000:1.

2.2. Nanowires, nanofibers and nanoprobes

Boron-doped silicon nanowires (SiNWs) were reported by **Cui et al. (2001)** to create highly sensitive, real-time electrically

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based sensors for biological and chemical species. The amine and oxide-functionalized SiNWs exhibited pH-dependent conductance that was linear over a large dynamic range and could be understood in terms of the change in surface charge during protonation and deprotonation. Biotin-modified SiNWs were used to detect streptavidin down to at least a picomolar concentration range. In addition, antigen-functionalized SiNWs showed reversible antibody binding and concentration-dependent detection in real time. The small size and capability of these semiconductor nanowires for sensitive, label-free, real-time detection of a wide range of chemical and biological species can be exploited in array-based screening and in vivo diagnostics.

The nanoscale size of these new class of sensors allows for measurements in the smallest of environments such as individual cells. This provides opportunities for in vivo monitoring of processes within live cells. **Cullum et al. (2000)** used optical fibers with a distal-end diameter of less than 1 Am, coated with antibodies, to detect the presence of toxic chemicals within single cells. They were able to measure the concentration of benzopyrene tetrol (BPT) within human mammary carcinoma cells and rat liver epithelial cells. **Tuan (2002)** fabricated nanoprobes with optical fibers pulled down to tips with the distal ends having sizes of approximately 30_50 nm **(Tuan,2002).** Using these nanobiosensors, it has become possible to probe chemical species at specific spots. Nanocontrolled release systems have been devised for optical biosensing of peroxide concentration **(Choi et al., 2001**).

2.3. Tubular and porous nanostructures

A common use of tubular and other porous nanostructures in biosensors is to increase the quantity and activity of the immobilized biomolecules. However, in view of their unique properties, these nanostructure provide opportunities for development of novel designs of biosensors. Use of the tubular and other porous nanostructures in biosensors is discussed next.

2.3.1. Carbon nanotubes

Since their discovery, carbon nanotubes have attracted great attentions as nanoscale building blocks for microdevices. The nano-dimensions, graphitic surface chemistry and electronic properties of carbon nanotubes make them an ideal material for use in chemical and biochemical sensing. Both single-wall nanotubes (SWNT) and multiwall carbon nanotubes (NWNT) have been used in biosensors (**Davis et al., 2003; Sotiropoulou et al., 2003)**.In one case, glucose oxidase was immobilized by coating onto the surface of singlewall nanotubes (SWNT) without a gross loss of enzyme activity **(Azamian et al., 2002).** The treatment of this bio-SWNT sensor with both a diffusive mediator and equilibrated glucose substrate enhanced the catalytic signal by more than one order of magnitude compared to that observed at an activated macro-carbon electrode. This enhanced performance was partly due to the high enzyme loading and partly because of better electrical communication ability of the nanotubes. The direct electron transfer ability of carbon nanotubes has been exploited in other cases. For example, use of SWNT has made possible a direct electron transfer with the redox active centers of adsorbed oxidoreductase enzymes **(Guiseppi-Elie et al., 2002).**

Both flavin adenine dinucleotide (FAD) and glucose oxidase (GOx) were found to spontaneously adsorb to unannealed carbon nanotubes that had been cast onto glassy carbon electrodes and to display quasi-reversible one-electron transfer. Similarly, GOx was found to spontaneously adsorb to annealed, single-walled carbon nanotube paper and to display quasi-reversible one-electron transfer. In particular, GOx immobilized in this way was shown to maintain its substrate-specific enzyme activity in the presence of glucose. It is believed that the tubular fibrils become positioned within tunneling distance of the cofactors without too much denaturation of the enzyme. The combination of SWNT with redox active enzymes appears to offer a convenient platform for a fundamental understanding of biological redox reactions and the development of reagentless biosensors and nanobiosensors. Similarly, horse radish peroxidase adsorbed on a carbon nanotube microelectrode was found to transfer electrons directly to the electrode and retain its catalytic activity toward H_2O_2 (Zhao et al., 2002). Carbon nanotube-based electrochemiluminescence (ECL) biosensors have been described. Wohlstadter et al. (2003) reported ECL biosensors for the assay of a-fetoprotein. Several characteristics make carbon nanotubes useful for ECL-based assays. Firstly, they are conducting, can act as electrodes, and can generate ECL signal. Secondly, they can be functionalized for the immobilization of biomolecules. In addition, carbon nanotubes have a high surface area-to-weight ratio and most of this surface area is accessible to both electrochemistry and immobilization of biomolecules. However, many of these functions can be just as effectively fulfilled by other noncarbon nanostructures such as metallic nanoparticles or fibers. Carbon nanotube array-based biosensors have been reported. Aligned multiwall carbon nanotubes (NWNT) grown on platinum substrate have been described for the development of an amperometric biosensor (Sotiropoulou et al., 2003). The two array systems in this work were either acid treated, or air treated. The results showed that chemical etching was more effective in opening the carbon nanotubes and allowing the enzyme to enter the inner channel. It seems that the oxidation of the array introduced carboxylic groups at the openends, to provide a stabilizing hydrophilic environment that allowed for the adsorption and insertion of the enzyme into the cavity of the nanotubes. Also, the immobilization of the enzyme within nanotubes may permit a mediated direct electron transfer to the platinum substrate transducer.

2.3.2. Other nanotube materials

Arrays of nanoscopic gold tubes have been prepared by electroless deposition of the metal within the pores of polycarbonate particle track-etched membranes **(Marc and Sophie, 2003)**. Glucose oxidase was immobilized onto the preformed self-assembled monolayers (SAMs) (mercaptoethylamine or mercaptopropionic acid) of gold tubes, via cross-linking with glutaraldehyde or covalent attachment by carbodiimide coupling. Glucose responses as large as 400 nA mM^{-1} cm^{-2} were obtained. Based on a slimier method of template synthesis**, Miao et al. (1999)** immobilized glucose oxidase in the polypyrrole nanotubes and produced a biosensor. Compared to conventional techniques, this immobilization strategy enhanced the amount of the enzyme immobilized, the retention of the immobilized activity and the sensitivity of the biosensor.

2.3.3. Porous silicon

Another nanostructure material that has been studied extensively for nanosensing applications is nanocrystalline silicon, often referred to as porous silicon. Since the discovery of its strong visible luminescence at room temperature, porous silicon has attracted considerable interest in its possible use in construction of biosensors. Its ability to emit light is due to its tiny pores that range from less than 2 nm to micrometer dimensions.

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In addition, porous silicon possesses a high surface to volume ratio (as much as 500 m2 cm_ 3) and it can be fabricated easily using some of the established processes of the usual silicon technology. Porous silicon has been used as an optical interferometric transducer for detecting small organic molecules (biotin and digoxigenin), 16-nucleotide DNA oligomers, and proteins (streptavidin and antibodies) at pico- and femtomolar analyte concentrations **(Lin et al.,1997; Di Francia et al., 1999).**

Microcavity resonators made of porous silicon have been used in biosensors. Theseresonators possess the unique characteristics of line narrowing and luminescence enhancement. **Chan et al. (2000)** fabricated a DNA biosensor based on a porous silicon microcavity structure. The microcavity structure was highly sensitive and any slight change in the effective optical thickness modified its reflectivity spectrum, causing a spectral shift in the interference peaks. Potentiometric biosensors based on porous silicon have been described **(Thust et al.,1996).** The enzymes penicillinase and lipase were separately immobilized on the surface of porous silicon to detect penicillin and triglycerides **(Scho¨ning et al., 2000; Reddy et al.,2001, 2003).** The hydrolysis reactions caused a change in the pH of the solution. The enzyme solution-oxidized porous silicon-crystalline silicon structure was used to detect the changes in pH during hydrolysis as a shift in the capacitance–voltage (C–V) characteristics. 2.4. Molecular self-assembly or biomimic based biosensors Molecular self-assembly mimics natural systems and is a key link between physics, chemistry and biology. Molecular self-assembly can be used to create novel structures, materials, and devices for use in biosensors (Nirmalya et al., 2002; **Boozer et al., 2003)**. Of all the self-assembled structures, thin lipid films and liposomes are the ones attracting the most attention in relation to biosensors (Dimitrios et al., 1999). Like a cell membrane, lipid films and liposomes are composed of phospholipids or other amphiphiles. Their hydrophilic/ hydrophobic characteristics allow them to spontaneously form organized structures. The supported bilayer lipid membrane (BLM) provides a natural environment for embedding proteins, receptors, membrane/tissue fragments, and entire cells under nondenaturing conditions and in a well-defined orientation. This makes BLMs specially attractive for use in biosensors. A successful biomimetically engineered device based on BLMs was the ion channel switch biosensor reported by **Cornell et al. (1997**). The basis of this 1.5 nm nanomachine was a self-assembled artificial membrane packed with gramicidin (Fig. 1). Ion channels were formed in the membrane by two gramicidin molecules: one in the lower layer of the membrane attached to a gold electrode and one in the upper layer tethered to biological receptors such as antibodies or nucleotides. The detection mechanism operated by binding the target molecule to the receptor and thereby altering the population of conduction ion channel pairs within the tethered membrane. This resulted in a change in the membrane conduction. The device was capable of detecting picomolar concentrations of proteins **(Woodhouse et al., 1999; Wright and Harding, 2000; Cornell et al., 2001).** Ambri (**http://www.ambri.com**) developed this technology into a commercial product, the SensiDxk system. This system can provide a broad menu of immunoassay, chemistry and other tests. The Ambri ICSk biosensor may haveapplications in healthcare, food, environment and other areas. Unlike planer BLMs, liposomes are microscopic, fluid-filled, pouches with endless walls that are made of layers of phospholipids identical to the phospholipids that make up cell membranes. Liposomes are typically used as the supporting substrate for immobilizing the biorecognition molecules. Liposomes are also used to amplify the optical, sound wave, and electrochemical signals **(Rongen et al., 1997; Hianik et al.,1999; Baeumner et al., 2003**). Polymerized lipid vesicles have been explored for developing smart colorimetric biosensors **(Kolusheva et al., 2001).** The vesicles are composed of three components: lipids, i.e. fat-like molecules that are the basis of normal biologic membranes; a specially designed lipid scaffold polymer known as a conjugated polydiacetylene (PDA); and membrane-soluble molecules containing epitopes (or protein fragments) that are recognized by the antibody under test. When inside the undisturbed vesicle, PDA gives the dispersion of vesicles a blue color. However, when an antibody is added that recognizes and binds the epitope, the vesicle is structurally distorted, causing the PDA to turn red. Polymerized lipid vesicles composed of PDA exhibit rapid colorimetric transition upon specific interactions with a variety of biological analytes and can be used to developed colorimetric biosensors. This simple approach that detects specific antibodies with a reagent that rapidly changes color can allow immediate diagnosis of diseases such as AIDS and tuberculosis. In addition, this approach can be used for colorimetric screening of enzyme catalysts, physiological ions, and the activities of antibacterial peptides (**Jelinek and Kolusheva,2001; Song et al., 2002).**

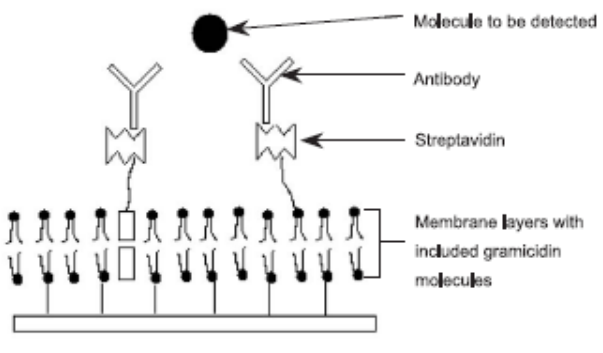

Fig. 1. The principle of the ion channel switch biosensor (http://www.ambri.com).

Fig. 1. The principle of the ion channel switch biosensor (http://www.ambri.com).

2.5. Nanofabrication

Nanofabrication uses integrated-circuit manufacturing technology and methods developed specifically for micromachining, to create nanometer size objects. Nanofabrication processes typically use variations of the four basic operations of photolithography, thinfilm growth/deposition, etching, and bonding. Nanoscaled interdigitated electrode arrays have been made with deep ultraviolet lithography (**Van Gerwen et al., 1998**). Electrode widths and spacings ranged from 500 to 250 nm on the active areas. Nanofabricated electrodes allow for the detection of affinity binding of biomolecular structures (e.g. antigens, DNA) by impedimetric measurements. For example, the immobilization of glucose oxidase could be monitored by measuring the double layer impedance. A sensitive conductimetric immunosensor was demonstrated based on an ultrathin platinum film on an oxidized silicon base **(Pak et al., 2001**). The film was about 25 A ° thick and consisted of a discontinuous layer with channels 20–30 A ° wide (Fig. 2). Impedance increased 55% at 20 Hz during the activation of the surface with anti-alkaline phosphatase (anti-AP) antibody. Binding of alkaline phosphatase (AP) to the prepared

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surface resulted in a further increase of 12% in impedance. p-nitrophenyl phosphate hydrolysis confirmed binding and activity of the AP. Modeling of thin-film response was used to distinguish between redox processes, capacitance and tunneling mechanisms. The data fitted well with the diffusion distributed elements model as well as a transmission line distribution element model. Nano-electromechanical system (NEMS) technologies are used to produce complex electrical, mechanical, fluidic, thermal, optical, and magnetic structures, devices, and
systems with characteristic sizes down to nanometers. NEMS creates and uses systems that have novel properties and functions because of their small and/or intermediate size. DNA hybridization and receptor–ligand binding to microfabricated cantilevers (Fig. 3)
produce surface stress changes that have been measured directly for detection of analytes(**http://www.zurich.ibm.com**) (**Fritz et al., 2000**). A biosensor is made by functionalizing one side of the cantilevers with receptor molecules and then detecting the mechanical bending induced by the binding of a ligand. Hybridization of complementary oligonucleotides with a single base mismatch between two 12-mer oligonoglobulin increased the differential signal by 10 nm, which was clearly detectable. Similar experiments on protein A–immunoglobulin interactions demonstrate a wide-ranging applicability of nanomechanical
transduction to detect biomolecular recognition. IBM researchers have further reported a microarray of cantilevers to detect multiple unlabeled biomolecules simultaneously at nanomolar concentrations within minutes (**McKendry et al., 2002).** This array permitted multiple binding assays in parallel and
could detect femtomoles of DNA on the cantilever at a DNA concentration in solution of 75 nM. The array of microfabricated cantilevers has been also functionalized with covalently anchored antibodies, to detect several different antigen–antibody reactions
simultaneously **(Arntz et al., 2003**). Gold nanoparticle modified DNA has been used to develop a microcantilever-based DNA biosensor (**Su et al., 2003**). The hybridization reactions led to the attachment of gold nanoparticles. Then gold nanoparticles acted as a nucleating agent for the growth of silver
particles when exposed to a photographic developing solution. The growth of silver particles increased the effective mass of the microcantilever and led to an enhanced frequency shift. This method could detect target DNA at a concentration of 0.05 nM or

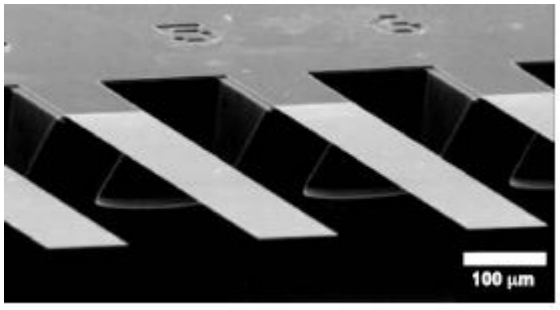

Fig. 2. An ultrathin platinum film immunosensor.

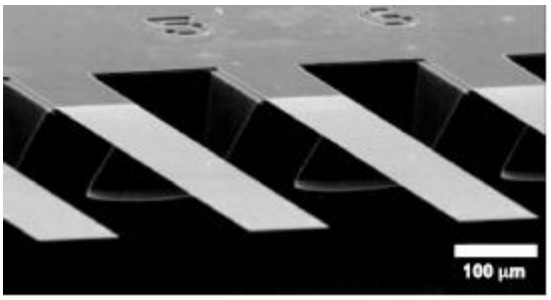

Fig. 3. Scanning electron micrograph of a section of a microfabricated silicon cantilever array (eight cantilevers,each 1 Am thick, 500 Am long, and 100 Am wide, with a pitch of 250 Am, spring constant 0.02 N m_1; Micro- and Nano-mechanics Group, IBM Zurich Research Laboratory, Switzerland) (Fritz et al., 2000).

lower. Combined with stringent washing, a single base pair mismatched DNA strand could be discriminated. Naval Research Laboratory, United States developed a force amplified biological sensor (FABS) capable of detecting biological species such as cells, proteins, toxins, and DNA at concentrations as low as 10_ 18 M (Fig. 4) **(Baselt et al., 1996). The** FABS design took advantage of the high sensitivity of force microscope cantilevers, to detect the presence of as few as one superparamagnetic particle bound to a cantilever by a sandwich immunoassay. The surface of the cantilever was coated with antibodies in the first step of
the sandwich assay. After the superparamagnetic beads were bound to the cantilever through immuno-interactions, the electromagnet was turned on. The magnetic field pulled on the beads, which pulled on the cantilever to cause it to bend. The bending was measured using piezoresistive cantilevers.

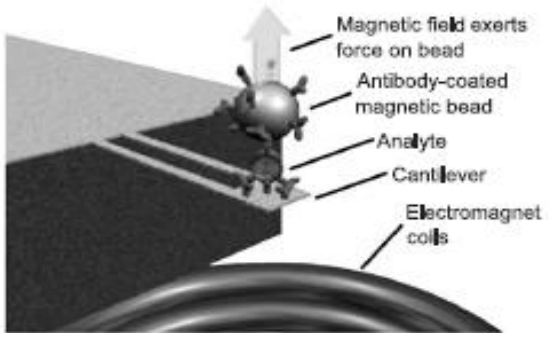

Fig. 4. FABS concept. A cantilever-beam force transducer senses the presence of magnetic beads, the number of which is proportional to the concentration of analyte in the sample. Many beads will typically attach to the cantilever (**Baselt et al., 1996**). Not to scale.

III. Conclusion

Nanotechnology is revolutionizing the development of biosensors. Nanomaterials and nanofabrication technologies are increasingly being used to design novel biosensors. Unfortunately, little attention is being given to the study of the various nanoeffects (e.g. quantum size effect, mini size effect, surface effect, macro-quantum tunnel effect) that are unique to nanomaterials and are actually their most attractive aspect. New nanomaterials and nanostructures need to be explored for use in biosensors. Preferably, nanotechnology-based biosensors should be integrated within tiny biochips with on-board electronics, sample handling and analysis. This will greatly enhance functionality, by providing devices that are small, portable, easy

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to use, low cost, disposable, and highly versatile diagnostic instruments.

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